AMENDMENTS TO THE SPECIFICATION

Please amend the specification by substituting the following re-written paragraphs:

On page 1, the unnumbered paragraph under the heading "Cross-Reference to Related Applications":

B1

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application Nos. ~~60/203,314 filed May 10, 2000;~~ 60/208,348 filed May 31, 2000; 60/208,111 filed May 31, 2000; 60/229,071 filed August 30, 2000; and 60/231,273 filed September 8, 2000.

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[0199] In the course of searching for what causes the growth of estrogen responsive breast and androgen responsive prostate cancers, it was discovered that the secretory immune system plays a major role in those diseases. More specifically, it was discovered that the secretory immune system (i.e., the immunoglobulins IgA, IgM and IgG1) provide negative (inhibitory) regulation of steroid hormone responsive mucosal epithelial cancer cell growth in serum-free model cell culture systems, including breast, prostate, pituitary, kidney, colon, and other glandular cancer cells. Prior to that discovery, which is described in co-owned concurrently filed U.S. Pat. App. No. 09/852,958 ~~(Atty. Dkt. No. 1944-00201)~~/PCT/US2001/15183 ~~(Atty. Dkt. No. 1944-00202)~~ entitled "Compositions and Methods for Demonstrating Secretory Immune System Regulation of Steroid Hormone Responsive Cancer Cell Growth," hereby incorporated herein by reference, no cell growth regulating role was known for the secretory immune system. The secretory immune system produces predominantly dimeric/polymeric IgA, secretory IgA (sIgA), polymeric IgM, and IgG1. The discovery of immunoglobulin inhibitors of cell growth is a major breakthrough in the understanding of cancers of breast and prostate, as well as other glandular/mucosal tissues that secrete or are bathed by the secretory immunoglobulins.

B3

[0204] The compositions and methods, and the biochemical, genetic and immunological tools described herein, and those described in U.S. Pat. App. No. 09/852,958 ~~(Atty. Dkt. No.1944-00201)~~/PCT/US2001/15183 ~~(Atty. Dkt. No. 1944-00202)~~ entitled "Compositions and Methods for Demonstrating Secretory Immune System Regulation of Steroid Hormone Responsive Cancer Cell Growth" (hereby incorporated herein by reference), are employed in the present investigations to further elucidate the cascade of cellular changes that lead to malignancy in glandular/mucosal tissues and to provide, among other things, ways of testing cancer cells for loss of IgA/IgM/IgG1 regulation, ways to detect genetic changes in the poly-Ig receptor, biochemical and genetic screening procedures to identify individuals at high risk for developing breast or prostate cancer, and ways of deterring or reducing the risk of development of such cancers.

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Additionally, in light of the discovery that the secretory immune system immunoglobulins IgA, IgM and IgG1 are potent inhibitors of steroid hormone responsive cancer cell growth, it is now proposed that the steroid hormone responsive tissues in the body can be protected from the cancer causing actions of certain environmental carcinogens, especially during age related "windows" of increased susceptibility, by enhancement of the IgA/IgM/IgG1 secreted by or coming in contact with those tissues. In this way, DNA synthesis dependent mutations can be prevented or substantially reduced in those tissues. Likewise, deleterious down-modulation or inactivation of critical gene expression (e.g., the poly-Ig receptor) due to environmental carcinogens may also be remediable by restoration of IgA, IgM and/or IgG1 control of cell growth.

B3

[0343] **Properties of the Serum-borne Inhibitor(s).** It is clear from the results presented herein, and described in co-owned, concurrently filed U.S. Patent App. No. 09/852,958 ~~(Atty. Dkt. No. 1944-00201)~~/PCT/US2001/15183 ~~(Atty. Dkt. No. 1944-00202)~~ entitled "Compositions and Methods for Demonstrating Secretory Immune System Regulation of Steroid Hormone Responsive Cancer Cell Growth," which is hereby incorporated herein by reference, that charcoal-dextran treated serum contains a sex steroid hormone reversible inhibitor(s) of target tumor cell growth in culture. This activity was identified as a progressive cell growth inhibition in culture medium containing 10% to 50% (v/v) hormone depleted serum. Despite its first proposal more than fifteen years ago, until the present invention, the inhibitor had yet to be purified, partially because of its instability. In an initial phase of investigations, a highly enriched fraction of serum protein was produced whose estrogen reversible inhibitory activity was stable and whose cell growth inhibitory effects replicate those seen with full serum with a variety of sex steroid hormone target tumor cell types in culture. Isolation was first attempted using an array of standard protein purification methods. Although they were expected to enhance stability, inhibitor activity was either not recovered after one only step or it was lost within two fractionation steps. In earlier work (Sirbasku DA *et al.* "Serum factor regulation of estrogen responsive mammary tumor cell growth." Proceedings of the 1997 Meeting of the "Department of Defense Breast Cancer Research Program: An Era of Hope", (Abstract) pp. 739-740, Washington, D.C., Oct. 31- Nov. 4, 1997) indicated that the inhibitor shared some properties with sex hormone binding globulin (SHBG). These results were obtained with a purification protocol known to simultaneously yield purified corticosteroid binding globulin (CBG) and SHBG from human cord serum (Fernlund P and Laurell C-B (1981) *J Steroid Biochem* **14**, 545-552). Additionally, it had been observed that the effect of calcium on both the estrogenic activity and the binding of ^{3}H- DHT to CDE-serum was remarkably similar to data presented by others concerning the stability of human SHBG (R sner W *et al.* (1974) *Biochim Biophys Acta* **351**, 92-98). Different laboratories have raised the issue of classical SHBG as

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the sex hormone reversible inhibitor of target cell growth. That protein binds both androgens and estrogens in plasma and acts as a carrier system with cell signaling characteristics (Rosner W (1990) *Endocr Rev* 11, 80-91). However, in view of the results presented herein and in U.S. Patent App. No. 09/852,958 ~~(Atty. Dkt. No. 1944-00201)~~/PCT/US2001/15183 ~~(Atty. Dkt. No. 1944-00202)~~, SHBG was considered an unlikely candidate for the inhibitor. Both CDE-horse serum and CDE-rat serum contain concentrations of inhibitor about equal to any of the other serum types investigated but they do not contain SHBG (Corvol P and Bardin CW (1973) *Biol Reprod* 8, 277-282; Renior J-M *et al.* (1980) *Proc Natl Acad Sci USA* 77, 4578-4582; Wenn RV *et al.* (1977) *Endokrinologie* 69, 151-156). Nevertheless, rabbit anti-human SHBG purchased from Accurate Chemicals not only immunoprecipitated the estrogenic activity in CDE-horse and rat serum, but also precipitated the ^{3}H-DHT (*i.e.* SHBG-like) binding activity in these sera. This coincidence initially led to the mistaken conclusion that the inhibitor was SHBG-like (Sirbasku DA *et al.* "Serum factor regulation of estrogen responsive mammary tumor cell growth." Proceedings of the 1997 Meeting of the "Department of Defense Breast Cancer Research Program: An Era of Hope", (Abstract) pp. 739-740, Washington, D.C., Oct. 31- Nov. 4, 1997). This misconception turned out to be fortuitous, however, as it led to a further exploration of the products obtained by the two-step cortisol agarose affinity and phenyl-Sepharose chromatography protocol. This protocol, when used with horse and rat serum, provided material that at concentrations of 10 to 15 µg/mL replicated the E_2 reversible inhibition caused by 30 to 50% (v/v) serum with steroid responsive human breast cancer cells, and responsive rat mammary, rat pituitary and Syrian hamster kidney tumor cells in culture. The inhibitor retained full activity for three years when stored unfrozen at -20°C in the presence of calcium, DHT and glycerol. As demonstrated herein, the long-standing problem of inhibitor instability has been overcome, and a highly active preparation became available to further probe molecular identity and mechanism(s) of action.

[0374] Cells in serum-free medium grow in response to nutrients, growth factors, metal delivery proteins, adhesion proteins, and various classes of hormones. All of these components are mitogenic in the sense that they contribute to cell replication. Nonetheless, the addition of only 20 µg/mL of inhibitor to block growth completely bears directly on the question of the progression of normal steroid target cells to fully hormone autonomous cancers. The inhibitor preparation used herein has the properties of a family of tissue regulators first named "chalones". These proposed cell regulators are water-soluble and tissue specific (but not species specific) proliferation inhibitors that are reversible by physiologic stimuli including hormones (Bullough WS (1975) *Life Sci* 16, 323-330; Finkler N and Acker P (1978) *Mt Sinai J Med* 45, 258-264). The studies presented herein support this classic concept

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as it applies to sex steroid hormone target tissues. The molecular identification of the serum inhibitor(s) promises not only to further support the role of estrogens as "necessary", but also to establish that "chalone-like" entities likely are the missing "sufficient" components that account for estrogen regulation of tissue growth. The application of serum-free defined medium conditions along with the use of a high specific activity fraction to demonstrate estrogen responsiveness in culture is unique. It should be noted that "chalones" have never before been identified. The results presented herein indicate, and in U.S. Pat. App. No. 09/852,958~~ (Atty. Dkt. No. 1944-00201)~~/PCT/US2001/15183~~ (Atty. Dkt. No. 1944-00202)~~ entitled "Compositions and Methods for Demonstrating Secretory Immune System Regulation of Steroid Hormone Responsive Cancer Cell Growth," hereby incorporated herein by reference, that the immune system is the long sought after source of these tissue specific inhibitors. In the series of studies described herein, the tissues are the mucosal tissues.

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[0456] **The Mediating Receptors – Inhibitory Function.** It has been made clear by the results presented herein, and in co-owned concurrently-filed U.S. Pat. App. No. 09/852,958~~ (Atty. Dkt. No. 1944-00201)~~/PCT/US2001/15183~~ (Atty. Dkt. No. 1944-00202)~~ entitled "Compositions and Methods for Demonstrating Secretory Immune System Regulation of Steroid Hormone Responsive Cancer Cell Growth," hereby incorporated herein by reference, that the mediating receptor for the serum-borne agent has special properties. As discussed above, serum contains a great variety of mitogenic agents. On this point, the present results in 50% (v/v) serum were especially relevant. This concentration of serum is a rich source of mitogens including insulin and the insulin-like growth factors. Nutrients and other serum components also have growth-promoting effects. Examples include diferric transferrin, unsaturated fatty acids bound to albumin, complex lipids and ethanolamine. The broad range of different "mitogens" present in defined medium are described elsewhere (Riss TL and Sirbasku DA (1987) *Cancer Res* **47**, 3776-3782; Danielpour D *et al.* (1988) *In Vitro Cell Dev Biol* **24**, 42-52; Ogasawara M and Sirbasku DA (1988) *In Vitro Cell Dev Biol* **24**, 911-920; Karey KP and Sirbasku DA (1988) *Cancer Res* **48**, 4083-4092; Riss TL *et al.* (1988) *In Vitro Cell Dev Biol* **24**, 1099-1106; Riss TL *et al.* (1988) *In Vitro Cell Dev Biol* **25**, 127-135; Riss TL and Sirbasku DA (1989) *In Vitro Cell Dev Biol* **25**, 136-142; Riss TL *et al.* (1986) *J Tissue Culture Methods* **10**, 133-150; Sirbasku DA *et al.* (1991) *Mol Cell Endocrinol* **77**, C47-C55; Sirbasku DA *et al.* (1991) *Biochemistry* **30**, 295-304; Sirbasku DA *et al.* (1991) *Biochemistry* **30**, 7466-7477; Sato H *et al.* (1991) *In Vitro Cell Dev Biol* **27A**, 599-602; Sirbasku DA *et al.* (1992)*In Vitro Cell Dev Biol* **28A**, 67-71; Sato H *et al.* (1992) *Mol Cell Endocrinol* **83**, 239-251; Eby JE *et al.* (1992) *Anal Biochem* **203**, 317-325; Eby JE *et al.* (1993) *J Cell Physiol* **156**, 588-600; Sirbasku DA and Moreno-Cuevas JE

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(2000) *In vitro Cell Dev Biol* 36, 428-446). From the present results, clearly, the immunoglobulin inhibitor(s) also block the growth effects of all those mitogens, and steroid hormones are selectively capable of reversing the effects of the inhibitor(s). Plainly, as predicted by the estrocolyone hypothesis, serum contains an inhibitor that has a dominant role in the regulation of proliferation of steroid hormone target cells. These inhibitors will have biological implications extending well beyond estrogen and androgen target tissues. Because of its "master switch" character, the newly identified immunoglobulin inhibitors have many practical industrial testing and manufacturing uses as well as many beneficial clinical applications.

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[0494] **Cell Growth Testing for Inhibitors.** In those cases where direct assessment of inhibitor in fluids is required, these can also be measured by cell growth assays on reduced microwell scale using automated colorimetric assays. The testing is carried out by first treating a plasma specimen to deplete or substantially remove the steroid hormone content without inactivating or removing the endogenous poly IgA dimer and poly IgM molecules. The hormone depleted specimen is then tested for cell growth inhibitory activity in the presence of added steroid hormone in an *in vitro* assay employing cultured tumor cells incubated in a defined serum-free medium. Procedures for preparing the steroid hormone depleted plasma or serum and for conducting the assay are described in preceding examples and in U.S. Pat. App. No. 09/852,958 ~~(Atty. Dkt. No. 1944-00201)~~/PCT/US2001/15183 ~~(Atty. Dkt. No. 1944-00202)~~ entitled "Compositions and Methods for Demonstrating Secretory Immune System Regulation of Steroid Hormone Responsive Cancer Cell Growth," hereby incorporated herein by reference. Application of the XAD-4™ resin treatment is preferred for small samples. These extraction methods are capable of yielding steroid hormone depleted serum that allows identification of 30 to 100-fold estrogen and androgen growth effects (cell number measurement) in culture in 7 to 14 days with human breast and human prostate cancer cells, as well at rat mammary, rat pituitary and Syrian hamster kidney tumor cells.

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[0575] As disclosed hereinabove and in U.S. Pat. App. No. 09/852,958 ~~(Atty. Dkt. No. 1944-00201)~~/PCT/US2001/15183 ~~(Atty. Dkt. No. 1944-00202)~~ entitled "Compositions and Methods for Demonstrating Secretory Immune System Regulation of Steroid Hormone Responsive Cancer Cell Growth," hereby incorporated herein by reference, the secretory immune system immunoglobulins IgA, IgM and IgG1 are potent inhibitors of steroid hormone responsive cancer cell growth in chemically defined serum-free medium. This serum-free cell culture system constitutes a preferred *in vitro* model of *in vivo* tumor cell growth that is superior to previously available serum-free systems. The inhibitory activity is mediated by poly-Ig receptor or a poly-Ig–like

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receptor. Among other things, this discovery has strong physiological significance in humans related to the well-known production of IgA, IgM and IgG1 in breast tissue and the secretion of these same immunoglobulins into breast milk. In the past, the IgA, IgM and IgG1 of milk were thought to serve only as an antibacterial protection for the suckling offspring. These same immunoglobulins, particularly in the form of polymeric IgA and pentameric IgM and IgG1, may also protect the mother and provide a new means of preventing or reducing her risk of breast cancer. Similar negative regulation by IgA, IgM and IgG1 has also been demonstrated by the inventor in androgen responsive prostate cancer cells. Analogous results are also indicated in steroid hormone responsive cancers of all other mucosal epithelial tissues that either secrete or are bathed by IgA, IgM and IgG1 in the body. These include not only tissues of the breast, prostate, pituitary and kidney, but also any other tissue that lines a cavity or secretes IgA/IgM/IgG1, such as tissues of the gastrointestinal tract (*i.e.* oral cavity mucosa, salivary/parotid glands, esophagus, stomach, small intestine and colon), tear ducts and nasal passages, liver and bile ducts, bladder, pancreas, adrenals, kidney tubules and glomeruli, lungs, the female reproductive tract (*i.e.* ovaries, fallopian tubes, uterus, cervix and vagina) and the secretory anterior pituitary gland. All of these glandular/mucosal tissues either secrete or are bathed by polymeric IgA, secretory IgA (sIgA), IgM and IgG1. Cancers arising from these tissues account for 80% of the epithelial malignancies of humans.

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[0586] Bacteria that meet the criteria described above will be cultured and the medium tested with non-tumorigenic human breast epithelial cells to determine if the cells are altered to a malignant phenotype. The test of altered growth will first be done in serum-free chemically defined medium, prepared as described the foregoing examples and in U.S. Pat. App. No. 09/852,958 ~~(Atty. Dkt. No. 1944-00201)~~/PCT/US2001/15183 ~~(Atty. Dkt. No. 1944-00202)~~ entitled "Compositions and Methods for Demonstrating Secretory Immune System Regulation of Steroid Hormone Responsive Cancer Cell Growth", or in Moreno-Cuevas and Sirbasku et al. (2000b), the disclosures of which are incorporated herein by reference. Transformed cells have reduced growth factor and adhesion requirements. Also, the cells will be tested for colony formation in standard assays. Normal epithelial cells will not form colonies in soft agar. Tumor or transformed cells will form colonies. There is a very strong correlation between colony forming activity in soft agar and tumorgenicity in host animals. These tests are expected to confirm that the mutagenic effects seen with the Ames Test can be translated to transformation of human breast cancer cells. Also, normal human prostate epithelial cells are available and will be used to perform a similar sequence of studies.

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